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AN INVESTIGATION OF THE RELIABILITY OF LABORATORY TESTS AND A DISCUSSION OF TECHNIQUE OF LABORATORIES IN AND NEAR BOSTON

by
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An Investigation of the Reliability of Laboratory Tests and a Discussion of Technique of Laboratories In and Near Boston

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FOREWORD BY DR. WM. C. WOODWARD, FORMERLY HEALTH COMMISSIONER, BOSTON, MASS.

IN May, 1919, the writer was impressed with the importance of determining the accuracy of diagnostic and other work done in the bacteriological laboratories in and about Boston. He had no reason to believe that conditions here were different from conditions in other places, but Metropolitan Boston is made up of so many independent cities and towns and contains so many clinical laboratories, public and private, in which bacteriological work is done, that the situation obtruded itself more forcibly, probably, than it would have done in other places. Action was taken toward the desired end by arranging for coöperation between the responsible directors of two bacteriological laboratories of unquestionable standing, with the hope that if coöperation between these two could be effected, then coöperation on the broader scale would follow. The plan was unsuccessful, however, the work consuming too large a part of the time of the directors of the laboratories participating in it.

Foiled in efforts along the line named, the writer appealed for aid to Professor William T. Sedgwick, then professor of biology and public health at the Massachusetts Institute of Technology. Professor Sedgwick cheerfully consented to direct the inquiry and to furnish the necessary technical assistance, but could not provide the money. An appeal was made to the Committee of the Permanent Charity Fund, Inc., who, upon being convinced of the importance of the objects to be obtained and of the fact that money was not available from other sources, made the necessary grant. The reasons submitted to the Committee of the Permanent Charity Fund in the appeal that was made for assistance seemed to represent a complete justification for the expenditure and were as follows:

"In the interest of the public the Health Department of the City of Boston, through its bacteriological laboratory, is continuously making examinations to determine whether persons from whom specimens are submitted by their attending physicians are or are not suffering from a communicable disease, to determine the nature and extent of the bacteriological contamination of milk, etc.

"It is manifestly of vital importance to the community and to the patient that the results of such examinations as are made to determine the presence or absence of communicable diseases be as nearly accurate as human care, knowledge, and skill can make them. To report that a smear from a case that a physician suspects of being a case of gonorrhoea shows that the case is that disease may create suspicions that no subsequent discovery of error and no amount of subsequent explaining can remove, and may wreck the happiness of a family. To report that a similar specimen shows no evidence of gonorrhoea when the question of marriage hangs on the decision may, if an error be made, ruin the health of the young wife and bring blindness to the offspring. Similarly the welfare of the individual and the safety of the community are inextricably bound up in the reports of the laboratory as to whether given cases are or are not syphilis, or tuberculosis, or diphtheria, or typhoid fever, or cerebro-spinal fever, or malarial fever, and so on.

"In the case of examinations made in connection with the efforts of the department to insure the wholesomeness of the milk supply, the safety of swimming pools, and so on, errors may lead to a false sense of security on the one hand, with consequent disaster, and on the other hand may lead to unnecessary expenditure of money, energy and time in efforts to right evils that do not exist."

The history and results of the investigation are fully set forth in the following report. Technical recommendations for the standardization and control of laboratory service, which are chiefly of interest to those engaged in laboratory work, are presented in a separate article for those interested in the purely laboratory aspects of the situation. To the administrative health officer and, above all, to the people, the matter of primary concern is not so much the standardization of procedure as the establishment of some method of supervision and control whereby laboratory errors and misinterpretation of laboratory reports will be reduced to a minimum.

It would seem clear that if physicians, pharmacists, dentists, veterinarians, and even chiropodists are required to satisfy the state of their fitness to follow their chosen callings before they are permitted to do so, then that persons who venture to presume that they are capable of doing clinical laboratory work should be tested in like manner before they begin to hold themselves out to the public, either in governmental or private laboratories, as qualified to do so; and some assurance should be required in every case that the laboratory equipment possessed by the would-be laboratory worker is adequate for the purposes for which he uses it. Possibly some such method of control, by requiring a license as a condition precedent to engaging in laboratory work as a means of livelihood, might be tolerated, for the present at least, inasmuch as that is the method of control applicable to the other callings named above. It would seem better even in such cases, however, and it would certainly seem necessary with respect to all governmental laboratories, that there be some adequate system of measuring the accuracy of their work from time to time and requiring that work to be up to standard. A peddler must produce scales of the required fineness before he can begin crying his wares, but, furthermore, he must have those scales tested from time to time to see that they retain their accuracy. So the public is entitled to demand that workers—at least in government laboratories—not only are qualified when they enter upon laboratory service but that they remain qualified during their entire period of service and that they do not fail through carelessness or rush of work to exercise their qualifications for the safeguarding of patrons of their laboratories.

To the end last named, there should certainly be established some system of measuring—of auditing, if you will—the accuracy of the reports issued from government laboratories. Such a system of checking up the work of these laboratories would not be difficult, provided only that money be provided for the employment of competent men for such work, with authority to use such measures as they saw fit for determining the accuracy of the results of any laboratory. Such work may well be entrusted to state medical licensing boards, or to state health authorities, but in the latter case, provision would have to be made for determining by some independent agency the accuracy of the work of the laboratories maintained by the state health authorities, which are certainly no more immune from error than are the laboratories in the municipal health departments. Possibly, after all, the establishment of an independent board for such supervisory work might best solve the problem.

Certainly, no fair-minded laboratory worker could object to such a system of supervision as is suggested above. It is just such a system of supervision as the most responsible financial interests of the country are by law subjected to, and such supervision as the financial departments of all large corporations expect from their stockholders and directors. If the work of the laboratory is above criticism, then an inquiry into it, such as is now proposed, will do the laboratory no harm and will tend to reassure the public and, particularly, the medical profession with respect to its work. If the work of the laboratory can be improved, then such supervision should show the way. Clearly it would be the part of wisdom on the part of state and municipal governments to provide such money as may be necessary, and to grant such authority as may be necessary, for the effective supervision and control of the laboratories established and maintained by them for the benefit of their citizens.

I cannot close without recording my appreciation of the valuable service rendered by Professor W. T. Sedgwick, Professor S. C. Prescott, Dr. L. S. Medalia, and Dr. F. H. Slack, and of the spirit of willing coöperation manifested by the several laboratories that were asked to coöperate in the investigation. I am under deep obligation, too, to the Committee of the Permanent Charity Fund, Incorporated, for the financial assistance rendered by it, without which the investigation would have been impossible. The investigation has, I feel, been well worth while, and it should point a way toward better laboratory organization and administration not only in Boston but wherever public health laboratories for the service of the community are maintained.

PART I.

A COMPARISON OF REPORTS FROM SEVERAL DIAGNOSTIC LABORATORIES ON FOUR COMMON TESTS: DIPHTHERIA, TUBERCULOSIS, TYPHOID FEVER, GONORRHOEA.

This report is a result of the initiative of Dr. Wm. C. Woodward, formerly Commissioner of Health for the City of Boston, through whom the Committee of the Permanent Charity Fund, Incorporated, became responsible for a study of the technique of various laboratories in or near Boston, a comparison of the work done, through the submitting of identical samples and an affort to establish uniform procedure.

The late Prof. W. T. Sedgwick of the Massachusetts Institute of Technology was asked by

Dr. Woodward to oversee this study and to assign workers from the Biology Department to prosecute it under his supervision. Under his direction Professor S. C. Prescott sent a circular letter to the various laboratories in March, 1920, and received a cordial response to his request for coöperation.

About June 1, 1920, Dr. L. S. Medalia took charge of the investigation and for several months devoted much of his time to a visitation of the laboratories and a study of the methods and technique of each.

On his request typewritten copies of the technique in use were submitted from the Boston Health Department laboratory, the State Health Department diagnostic laboratory, the State Wassermann laboratory and from the Board of Health laboratories in Brookline, Cambridge and Somerville.

Dr. Medalia drew up charts comparing the technique in use in these and other laboratories for the diagnosis of diphtheria, tuberculosis and typhoid fever. He also called several meetings of laboratory directors for a discussion of the technique used in these tests.

In May, 1921, owing to the pressure of other work, Dr. Medalia felt obliged to drop this investigation, and the writer was asked to continue it.

Following the programme outlined for the work, this was done by submitting various series of specimens to the laboratories for diagnosis, a comparison of the reports and repeated conferences of the workers for discussion of technique. As an outcome of the discussion a code of minimum standards has been accepted.

The writer acknowledges with appreciation the hearty coöperation of the following laboratories in carrying out these tests, including, besides those mentioned in this paper, Wassermann tests and examination of milk: Boston Biochemical Laboratories, Boston City Hospital Pathological Laboratory, Boston Dispensary Laboratories, Boston Health Department Bacteriological Laboratory, Boston Health Department Bureau of Milk Inspection, Brockton Health Department Laboratories, Brookline Health Department Laboratory, Cambridge Health Department Laboratory, Homeopathic Hospital Pathological Laboratory, Massachusetts General Hospital Pathological Laboratory, Massachusetts State Department of Health Bacteriological Laboratory, Massachusetts State Department of Health Wassermann Laboratory, Newton Health Department Laboratory, Newton Hospital Laboratory, Peter Bent Brigham Hospital Pathological Laboratory, Wellesley Health Department Laboratory.

DIPHTHERIA.

Through the courtesy of Dr. E. H. Place of the Boston City Hospital, South Department, the first series of diphtheria cultures was taken from patients and attendants there. Ten cultures were taken. The swabs in each case were then rubbed thoroughly over the surface of as many tubes of blood serum media as there were laboratories taking part, and to make sure of a thorough inoculation the process was repeated. The last serum in each case, which presumably received the least material, went to Laboratory No. 7. All the serums were delivered to the various laboratories on the same day.

The results of this first series of tests were rather disappointing, evidently where the bacilli were few, as in Nos. 1 and 3, they were either overgrown or overlooked in over 50 per cent. of the cultures.

Brief histories of these ten cases follow:

Case No. 1. No. 64724. Age five years. Admitted April 15, 1921. *Diagnosis:* Clinical nasal diphtheria. *P. E.:* Bilateral muco-purulent discharge from nose with crusting. No membrane seen. Positive culture from nose and throat at entrance. *Course:* Received antitoxin at entrance. Uneventful course till May 21, when adenoids were removed. Throughout convalescence positive cultures were obtained until June 10, when gentian treatment was used for one week and then omitted for one week, subsequent to which all cultures from nose and throat were negative. Virulence test not done. *Diagnosis:* Nasal diphtheria.

Case No. 2. No. 64755. Age six years. Admitted May 11, 1921. *Diagnosis:* Typical mild faucial diphtheria. Positive cultures from the nose and throat. Received antitoxin at entrance. *Course:* Uneventful convalescence. Was discharged well at the end of three weeks. Discharge cultures all negative. *Diagnosis:* Faucial diphtheria.

Case No. 3. No. 64734. Age eight years. Admitted April 18, 1921. *Diagnosis:* Typical clinical faucial diphtheria, moderate. Positive culture from throat. Received antitoxin at entrance. *Course:* Uneventful convalescence, with discharge in twenty-four days. All discharge cultures negative. *Diagnosis:* Faucial diphtheria.

Case No. 4. No. 64859. Age six years. Admitted May 7, 1921. *Diagnosis:* Faucial diphtheria. Severe toxaemia. Entrance cultures positive. Forty thousand units antitoxin given at entrance intravenously. History not obtained. Duration of illness not known. *Course:* There was considerable difficulty in breathing due to oro-pharyngeal obstruction, which was relieved by passing a nasal tube. There was a slight febrile disturbance due to antitoxin, with temperature returning to normal in twelve hours. Death followed on fifth day in hospital. *Diagnosis:* Faucial diphtheria.

Case No. 5. No. 64873. Age twenty-one years. Admitted May 10, 1921. *Diagnosis:* Mild faucial diphtheria. Positive culture from

DIPHTHERIA TESTS—TABLE I.

CULTURES TAKEN MAY 11, 1921.

	Specimens.									
Lab.	1	2	3	4	5	6	7	8	9	10
1	—	—	—	—	+	—	+	+	+	—
2	—	—	—	c*	+	+	—	+	+	—
3	+	—	—	—	+	+	—	+	+	—
4	—	—	—	c	+	+	Susp.	+	+	—
5	—	—	—	c	+	—	—	—	+	+
6	+	—	+Few	—	+	+Few	+	+	+	—
7	—Susp.	—	+Few	c	+	+	+	+	+	—
8	—?	—	—?	c	+	+	+	—?	+	—
9	—??	—	—?	c	—?	—?	+?	—	+???	—
10	—?	—	—?	c	+	+?	+	—?	+	—
11	+	—	+	—	+	+	+	+	+	—
12	—	—	—	—	+	—	+	+	+	—

*c=contaminated.

throat. Received antitoxin at entrance. *Course*: Uneventful. Discharged in eighteen days. All release cultures negative.

CASE No. 6. No. 64688. Age seven years. Admitted April 11, 1921. *Diagnosis:* Mild nasal diphtheria? *Course:* No membrane in nose. Entrance cultures negative three successive days from nose and throat separately. Antitoxin given at entrance. Uneventful course till placed on release cultures April 26. From 26th of April to 4th of May all release cultures were negative, but patient did not go home. From that date on, positive cultures were noted almost constantly. From May 15 to 23 gentian treatment was given. From May 28 cultures were again positive persistently from the nose and occasionally from the throat. Adenoidectomy June 3. Cultures from June 8 to 15 positive from nose and throat. Virulence test proved organism was non-virulent. *Discharge:* Patient discharged well July 16, 1921. *Diagnosis:* Nasal diphtheria.

CASE No. 7. No. 64435. Age eight years. Admitted March 4, 1921. *Diagnosis:* Mild faucial diphtheria. Positive culture from throat at entrance. Antitoxin given. *Course:* Uneventful save for otitis media. Release cultures from nose, throat and ears were negative March 17 to 21 and patient was discharged March 22. After discharge one positive culture from nose and throat returned. Patient was readmitted March 24, No. 64556, with positive culture from the nose. No antitoxin given, no Schick done. Uneventful course save for almost continuous positive cultures. Gentian treatment in April followed by positive cultures again. Regional cultures showed nose alone involved. June 3 tonsils and adenoids removed; following day had a moderately bad post-nasal hemorrhage. Cultures resumed June 10; positive till the 15th; negative through the 21st. Discharged 22d of June well. *Diagnosis:* Faucial diphtheria, tonsils and adenoids.

CASE No. 8. No. 64847. Age twelve years. Admitted May 6, 1921. *Diagnosis:* Severe faucial diphtheria. *P. E.* Patient admitted with severe faucial diphtheria with oedema of soft palate and considerable membrane. Antitoxin given at entrance. *Course:* Cultures from throat and nose, suspicious for several days, became definitely positive fourth day after entrance. Course uncomplicated save for slight evidence of kidney inflammation. Definite palatal paralysis, also knee jerks. Release cultures begun June 24 were all negative. Discharged well June 30, 1921. *Diagnosis:* Faucial diphtheria.

CASE No. 9. No. 64664. Age twenty-one years. Admitted May 9, 1921. *Diagnosis:* Streptococcus sore throat. Patient admitted with streptococcus sore throat of moderate severity. Cultures from nose and throat separately negative. Received antitoxin at entrance. *Course:* Moderate serum reaction. Out of bed April 29. Uneventful course save for occasional positive cultures among discharge cultures from April 23 to May 14, when the regional cultures showed tonsils and posterior pharynx alone involved. Then came T. and A. May 21, with resumption of cultures May 28. From May 28 to June 8 all cultures for release except the one on June 1 were negative. Patient discharged well June 9. *Diagnosis:* Streptococcus sore throat. Tonsils and adenoids removed. Bacteriological diphtheria.

CASE No. 10. No. 64855. Age four years. Admitted May 7, 1921. *Diagnosis:* Measles. *Course:* Entrance culture from nose positive. No antitoxin given. Schick negative. Uneventful convalescence. Release cultures from May 9 to 17 all negative. *Discharge:* Patient discharged well May 15, 1921. *Diagnosis:* Measles.

As indicated in the histories, many of these were release cultures, and on this account more difficult for examination. Most of the errors consisted in passing as negative cultures containing but few bacilli. Laboratory No. 5 reports a positive result on culture 10, which all others report negative. Laboratory No. 9 seems over-cautious.

In preparing the material for the second series of diphtheria tests the duplicate smears were made in each case from a single culture, the growth having been first emulsified in salt solution. These smears, dried on glass slides, were submitted to the laboratories, each of which was allowed to stain the specimens in its own way.

DIPHTHERIA TESTS—TABLE II.

	Specimens.				
Lab.	1	2	3	4	5
1	+	—	—	—	?
2	+	—	—	—	—
3	—	—	—	—	+
4	+	—	—	—	+
5	—?	—	—	—	+
6	+	—	—	—	?+
7	+	—	—	—	+
8	+	—	—	—	?
9	+Few	—	—	—	+
10	No answer received				
11	+	—	—	—	—

Cultures Nos. 1 and 5 were positive, the others negative. All agree on the negatives, and, again, the chief error is in reporting as negative smears containing diphtheria bacilli.

It is interesting to note that while each laboratory reported one or the other of these cultures, 1 and 5, as positive, only three give a positive report on both.

One laboratory did not answer at all, and a troublesome feature in all these tests was the slow progress because of delay in sending reports.

The writer realizes that these tests on diphtheria are too few for a basis for comment, and is planning another series of tests in which the same stained smears will be submitted to all laboratories, thus eliminating discrepancies due to varying cultural conditions or to methods of preparation of the specimens.

It seems evident that positive cultures, containing few bacilli, are apt to be reported negative, especially by the smaller laboratories. Larger laboratories have many advantages which tend to give them greater accuracy. In the first place, the handling of numerous cultures daily keeps the technician in good training, and, secondly, the larger staff allows for the examination of each specimen by at least two observers.

TUBERCULOSIS.

Only one series of specimens has been submitted, and these were smeared and fixed on glass slides, each laboratory doing its own staining.

SPUTUM EXAMINATION FOR T.B.

Lab.	1	2	3	4
1	+	—	—	+
2	+	—	—	+
3	+	—	—	+
4	+	—	—	+
5	+	—	—	+
6	+	—	—	+
7	+	—	—	+
8	+	—	—	—
9	+	—	—	+Few
10	Did not answer			
11	+	—	—	+

Only one mistake is noted here: a case of overlooking the bacilli when they are few. This is the chief error to be expected in this examination.

TYPHOID FEVER.

For this test specimens of dried blood were submitted, the positives from a guinea pig which had been immunized, the negatives from a non-immune pig. Specimens 1, 2 and 5 were negative.

The specimens were submitted in July.

WIDAL TESTS.

Lab.	1	2	3	4	5	
1	—	—	+	+	—	Reported Sept. 8
2	—	—	+	+	—	Reported in July
3	—	—?	+	+	—	Reported in July
4	—	—	+	+	—	Reported in July
5	—	—	+?	+?	—	Reported in July
6	+	—	+?	+	—	Reported Sept. 17
7	No report received					

The principal criticism here is in the delay of the reports. One negative specimen was reported as positive by Laboratory No. 6.

GONORRHOEA.

Four smears were submitted to each laboratory, being prepared through the kindness of Dr. Hinton of the Boston Dispensary from four patients, two with positive and two with negative discharges.

GONORRHOEA TESTS.

Lab.	1	2	3	4
1	—	—	+	+
2	—	—	+	+
3	—	—	+	+
4	—	—	+	+
5	—	—	+	+
6	—	+	+	+
7	—	—	+	+
8	—	—	+	+
9	—	—	+	+
10	—	—	+	+
11	—	?	+	+
12	—	—	+	+

One of the negative smears was reported positive by one laboratory and suspicious by another. One great difficulty in gonorrhoea diagnosis is with the proper control of the gram stain. Unless considerable care is used one may decolorize too much, causing gram-positive organisms to appear as gram-negative; or one may decolorize too little, causing gram-negative organisms to appear as gram-positive.

Since one laboratory reported this difficulty overcome by use of Burke's staining method, the formula for this method will, at the request of the other laboratories, be included in the report on technique.

Of these four examinations diphtheria is undoubtedly the most difficult, and the results in the other three series (tuberculosis, typhoid fever and gonorrhoea) are fairly uniform. With diphtheria, however, the discrepancies are very noticeable, and this in spite of the fact that the various workers knew their results were to be compared with those obtained by others.

Since all the diphtheria cultures, with the exception of sets for two laboratories*, were planted on tubes from the same batch of media, and all were delivered to the laboratories within a few hours, it would seem that the cultures should be uniform, yet in one case reported positive by the writer a smear from a duplicate culture was brought to him which showed no diphtheria bacilli.

Probably cultures for diagnosis would have given better results, since the bacilli are apt to be more numerous and of better morphology.

One cannot but feel, however, that diphtheria bacilli have been overlooked in some of these cultures and that some of the technicians need toning up on this examination.

Possibly a frequent comparison of duplicate cultures with other laboratories, or occasional revision of diphtheria slides, staining methods and diagnoses by an expert diagnostician, would be of advantage in establishing a higher precision in diphtheria examinations.

PART 2.

WASSERMANN TESTS — A COMPARISON OF REPORTS FROM SEVERAL LABORATORIES ON IDENTICAL SPECIMENS.

The method first adopted for comparing Wassermann tests was to pool the left-over serums from positive, negative or doubtful cases and then divide into a sufficient number of specimens to supply the laboratories. The first series had two positive, two negative and two doubtful specimens.

Here there is complete agreement on strong positives and on straight negatives. Border line serums receive reports varying from positive to negative, depending, probably, on the strength of the hemolytic system used.

WASSERMANN TESTS—FIRST SERIES.

	Specimens.				
Lab.	1	2	3	4	5	6
1	—	+	+	—	—	—
2	Doubtful	+	+	—	—	Broken tube
3	—	+	+	—	—	—
4	±	++++	++++	—	—	+++
5	—	+	+	—	—	Mod. Pos.
6	—	+	+	—	—	—
7	—	+	+	—	—	—?

WASSERMANN TESTS—SECOND SERIES.

Lab.	1	2	3	4	5	6
1	+	—	Uns.	Doubt	Uns.	—
2	+	—	+	+	+	—
3	+	—	hem.	hem.	+	—
4	a. c.	—	a. c.	a. c.	a. c.	—
5	+	—	—	—	+	—
6	+	—	+	Doubt	+	—
7	+	—	a. c.	a. c.	+	—

Serums No. 1 and 5 were identical from pooled positive serums. Laboratory No. 1 reports 1 positive and 5 unsatisfactory. Laboratory No. 4 reports both anticomplementary, and inquiry indicates that Laboratory No. 4 is using an insufficient dose of complement.

Serums No. 2 and 6 from pooled negatives all agree upon. Serums No. 3 and 4 are identical from pooled doubtful serums. Reports on these vary from negative through doubtful and anticomplementary and hemolysed to positive. Laboratory No. 6 reports No. 3 positive and No. 4 doubtful.

WASSERMANN TESTS—THIRD SERIES.

Lab.	1	2	3	4	5	6
1	—	—	+	+	+	—
2	—	—	+	+	+	—
3	—	—	+	+	+	a. c.
4	—	—	+	+	a. c.	—
5	—	—	+	+	a. c.	—
6	—	—	+	+	+	—
7	—	—	+	+	a. c.	—
8	—	—	+	+	+	—

Specimens 1, 2 and 6 are identical. All agree except an a. c. report by Laboratory No. 3 on specimen 6. Specimen No. 5 was from pooled doubtful serums. It seems the pooled serums gave a stronger reaction than did the individual ones, since five out of eight laboratories report this specimen positive.

On the whole, the indications from these three series of tests seem to be that with a strong positive or with a straight negative specimen reports from reputable laboratories will agree, while with a weakly positive or doubtful specimen the report will vary from negative to positive, depending on the individual adjustment of the hemolytic system.

Comparative Tests with Specimens from Individuals.—In the discussion of the work done on pooled serums differences of opinion arose which, it seemed, might be cleared away by running a series of tests on serums from individuals where the history was known. Dr. Hinton kindly agreed to furnish these serums from the laboratory of the Boston Dispensary.

Besides the Boston Dispensary laboratory, the following laboratories joined in the tests: Boston City Hospital, Boston Health Department, State Wassermann laboratory, Homeopathic Hospital Laboratory and the Sias Laboratory.

The serums were distributed Thursdays and tests were made Fridays, excepting in the Dispensary, which made the tests a day earlier.

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*One of these laboratories preferred to reinoculate from the cultures submitted to its own media; the other made its own cultures from the swabs.

In this series of forty-three tests there were twenty-three on which all the reports agree as negative; twenty of these have no history of syphilis; the others, Nos. 9, 22 and 31, are cases of treated syphilis.

There were five on which all agree as positive, Nos. 1, 3, 24, 25 and 30, all giving histories of syphilis.

There were seven on which there is practically agreement in the report of negative, the minority reporting doubtful or doubtfully weak positive, which the technician of the latter laboratory explains might as well be reported doubtfully negative,—Nos. 4, 8, 16, 17, 19, 34 and 36. Four with a negative history, two of treated syphilis and one where there is doubt.

There were two on which there is practically agreement on the report of positive, a minority worker reporting doubtful in one case and anticomplementary in the other. These are Nos. 11 and 32, both with histories of syphilis.

Thus, of the forty-three cases, or 258 tests, there remain but six in which there are any real disagreements.

Case No. 5, reported positive by five workers, negative by one, gives a history of syphilis, with at least twelve arsphenamines.

Case No. 7, reported positive by four, anticomplementary by one, negative by one, gives no history of syphilis, and was thought probably tubercular. In view of the Wassermann findings, the case is probably syphilitic.

Case No. 12, reported positive by four, doubtful by one, negative by one, has a history of two miscarriages and two arsphenamine treatments.

Case No. 13, reported positive by three, doubtful by one, (?) negative by one, and negative by one, gives a history of syphilis with thirty to forty arsphenamines.

Case No. 38, reported positive by three, doubtful by two, negative by one, gives a history of syphilis, with six positive and three negative Wassermanns.

Case No. 42, reported negative by four, doubtful by one, positive by one, is evidently from the history a case of treated syphilis.

HISTORIES.

1. (14566)—Man, chancre twenty-five years ago. Treated for five weeks. Positive Wassermann.

2. (14585)—Janitor, sixty-six years old, who states there is no history of gonorrhoea or chancre. Negative clinically. Negative Wassermann. Diagnosis, flat-foot.

3. (14565)—Chancre sixteen years ago. Treated for one and a half years. Has had more than seventeen arsphenamines in the last three years, together with mercury and iodides. Previous Wassermanns—positive, doubtful, doubtful, negative, positive, positive. Diagnosis syphilis with tabetic symptoms.

4. (14583)—Jewish woman of twenty-seven with a diagnosis of tendon strain and no history of syphilis. Negative clinically. Negative Wassermann. Diagnosis, tendon strain.

5. (14548)—Storekeeper, forty-eight years old, denying chancre and other symptoms referable to syphilis and giving history of tuberculosis for four and a half years, now inactive. Three positive Wassermanns followed by a doubtful and later a positive. During the course of these Wassermann tests has had at least twelve arsphenamines covering a period of three months. Diagnosis, not stated, but patient is now being given syphilitic treatment.

6. (14580)—Girl of eleven, whose parents are syphilitic. Three negative Wassermanns for a period of three months. Diagnosis, the Wassermann tests taken to determine the presence of syphilis on the basis of parentage.

7. (14574)—Girl of eighteen years without stigmata of syphilis, showing ulcerated areas over the buttocks and over the arms, which are of questionable origin. Tuberculosis considered. Diagnosis, question of lupus.

8. (14588)—Married woman of twenty-four without history of syphilis. Wassermann negative. Diagnosis deferred.

9. (14559)—A married Greek woman of thirty-two who has been treated for syphilis here since 1916, having had a lot of salvarsan, mercury and iodides. Seven Wassermann reactions, all negative. Diagnosis, treated syphilis.

10. (14578)—Jewish woman of fifty without history of syphilis and a clinical diagnosis of torticollis. Wassermann negative.

11. (14595)—Child of syphilitic mother. Two positive Wassermanns. Amount of treatment prior to Wassermann tests not shown in the record.

12. (14546)—Jewish widow thirty years of age with a history of two miscarriages. Positive Wassermann in New York and two injections of arsphenamines. Gives no history of primary or secondary lesions. Wassermann positive.

13. (14555)—Married man of forty-four, infected sixteen years ago. Negative Wassermann, doubtful Wassermann, lumbar puncture spinal fluid positive Wassermann, cells twenty-seven, positive gold sol. reaction, doubtful Wassermann, negative Wassermann, two positive Wassermanns, negative Wassermann. Had had thirty or forty arsphenamines. Diagnosis, syphilis; tabo-paresis.

14. (14568) — Married American porter, forty years of age, treated for gonorrhoea. At present has acne. Wassermann negative.

15. (208)—Married Jewess, thirty years of age, without symptoms or physical examination of syphilis. Wassermann negative. Diagnosis deferred.

16. (191) — Married female, Portuguese, twenty years of age, with periostitis of the

No. of Specimen.		LAB. A.					LAB. B.				LAB. C.					LAB. D.				LAB. E.				LAB. F.			
		State antigen.	Chol. antigen.	Ac. Ins. with 1/10 serum.	Ac. Ins. with 4/10 serum.	Report.	State antigen.	Ac. Ins. with 1/10 serum.	Ac. Ins. with 4/10 serum.	Report.	Chol. antigen 1 serum 0.1.	Antigen 1 serum 0.05.	Chol. antigen 2 serum 0.1.	Antigen 2 serum 0.05.	Report.	G. pig heart antigen.	Human heart antigen.	State antigen.	Report.	G. pig heart antigen.	Human heart antigen.	State antigen.	Report.	State antigen.	Chol. antigen.	Control.	Report.
14566	1	+	+	+	+	Strong Pos.	+	+		Pos.	4	4	4	4	Strong Pos.	+	+	+	Pos.	0	+	+	Strong Pos.	4	4	—?	Strong Pos.
14585	2	—	—	—	—	Neg.	—	—		Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.
14565	3	+	+	—	+	Mod. Pos.	+	—	+	Mod. Pos.	4	±	4	±	Mod. Pos.	+	+	+	Pos.	+	0	+	Strong Pos.	4	4	—	Strong Pos.
14583	4	+	—	—	—	Neg.	—	—		Neg.	2	—	±	—	Doubt Weak Pos.	—	—	—	Neg.	—	0	—	Neg.	4	3	—	Mod. Pos.
14548	5	+	+	—	+	Mod. Pos.	+	—	+	Mod. Pos.	4	1	4	2	Mod. Pos.	+	+	+	Pos.	w +	w +	—	Neg.	4	4	—	Strong Pos.
14580	6	—	—	—	—	Neg.	—	—		Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	1—	—	—	Neg.
14574	7	+	+	+	+	Strong Pos.	+	+		Pos.	4	4	4	4	Strong Pos.	—	—	—	Neg.	0	+	+	Strong Pos.	4	4	4	Ac.
14588	8	+	—	—	—	Neg.	—	—		Neg.	1	—	±	—	Doubt. Weak Pos.	—	—	—	Neg.	—	w +	—	Neg.	4	—	—	Neg.
14559	9	—	—	—	—	Neg.	—	—		Neg.	±	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	2—	—	—	Neg.
14578	10	—	—	—	—	Neg.	—	—		Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	—?	—	—	Neg.
14595	11	+	+	+	+	Strong Pos.	+	+		Pos.	4	4	4	4	Strong Pos.	+	+	+	Pos.	+	0	+	Strong Pos.	4	4	4	Ac.
14546	12	+	+	—	±	Doubt.	—	—		Neg.	4	3	4	3	Mod. Pos.	+	+	+	Pos.	0	+	+	Strong Pos.	4	4	—	Strong Pos.
14555	13	+	+	—	—	? Neg.	+	—	—	Doubt.	4	±	4	±	Mod. Pos.	—	—	—	Neg.	+	0	+	Strong Pos.	4	3—	—	Mod. Pos.
14568	14	+	—	—	—	Neg.	—	—		Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	2	—	—	Neg.
0208	15	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	—	Neg. Doubt,	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.

0191	16	—	—	—	—	Neg.	—	—	—	Neg.	±	—	±	—	W. Pos.	M	—	—	Doubt.	—	0	—	Neg.	—	—	—	Neg.
0193	17	+	—	—	—	Neg.	—	—	—	Neg.	2	—	2	±	Doubt. w. Pos.	—	—	—	Neg.	—	—	—	Neg.	2	—	—	Neg.
0233	18	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.
0206	19	+	—	—	—	Neg.	+	—	—	Doubt.	3	±	3	±	Weak Pos.	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.
0242	20	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.
0243	21	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.
0197	22	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.
0216	23	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.
0214	24	+	+	+	+	Strong Pos.	+	W +	+	Pos.	4	3	4	3	Mod. Pos.	+	+	+	Pos.	+	m +	m +	Pos.	4	2	—	Mod. Pos.
0194	25	+	+	+	+	Strong Pos.	+	m +	+	Pos.	4	4	4	4	Strong Pos.	+	+	+	Pos.	+	+	+	Pos.	4	4	—	Strong Pos.
0238	26	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.
0244	27	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.
0236	28	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.
0234	29	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.
0382	30	+	+	+	+	Strong Pos.	+	+	+	Pos.	4	4	4	4	Strong Pos.	+	+	+	Pos.	+	+	+	Pos.	4	4	—	Pos.
0386	31	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.
0384	32	+	+	—	+	Mod. Pos.	+	+	+	Pos.	4	4	4	4	Strong Pos.	w +	m +	m +	Doubt.	+	+	+	Pos.	4	4	—	Strong Pos.
0394	33	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.
0385	34	—	—	—	—	Neg.	±	—	±	Doubt.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.
0383	35	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.
0398	36	—	—	—	—	Neg.	m +	—	—	Doubt.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	1	—	—	Neg.
0410	37	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.
0389	38	+	±	—	+	Mod. Pos.	+	—	+	Mod. Pos.	4	2	4	±	Mod. Pos.	—	—	—	Neg.	+	—	—	Doubt.	4	1	—	Doubt. Weak Pos.
0396	39	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.
0388	40	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.
0415	41	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.
0390	42	—	—	—	—	Neg.	+	—	—	Doubt.	3	1	3	1	Mod. Pos.	—	—	—	Neg.	—	—	—	Neg.	3	—	—	Neg.
0411	43	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	—	Neg.	—	—	—	Neg.	—	—	—	Neg.	2	—	—	Neg.

radius and enlarged epitrochlears, who has had six doses of salvarsan at the Boston Dispensary. Wassermann doubtful. Diagnosis, treated syphilis.

17. (193)—Italian farm hand, seventy years of age, without history or symptoms of syphilis. Wassermann negative. Diagnosis, epithelioma of the cheek.

18. (233) — Lithuanian married woman, thirty-nine years of age, who has had two miscarriages, but without any other symptoms referable to syphilis. Wassermann negative. Diagnosis deferred.

19. (206)—Italian bricklayer, forty-eight years of age, who comes with a history of having been treated for syphilis in Hartford four years ago. No physical examination taken. Wassermann negative.

20. (242)—Married woman of twenty-five, has had a medical examination; no history nor clinical evidence of syphilis. Wassermann negative. Diagnosis, psychasthenia.

21. (243)—Married Italian woman, twenty-three years of age, without history or clinical evidence of syphilis. Wassermann negative. Diagnosis, chronic pharyngitis, question of proctitis.

22. (197)—Female, thirty-eight years of age, with a history of syphilis dating back as late as seven years, who has had numerous doubtful and a few negative Wassermanns, and who has had a lot of anti-syphilitic treatment, including arsphenamines. Diagnosis, treated syphilis.

23. (216)—American, thirty-nine years of age, complaining of a rash of four days' duration; also presents a penile sore of four days' duration; dark field shows no pallida. No previous evidence of syphilis. Wassermann negative. Diagnosis deferred.

24. (214) — Married American Negress, twenty-three years of age, with a history of primary seven years ago. Five positive Wassermanns; large amount of syphilitic treatment. Diagnosis, treated syphilis.

25. (194)—Fireman, twenty-eight years of age, with a history of chancre a year ago. Amount of treatment not indicated on the record. Wassermann positive. Diagnosis, treated syphilis.

26. (238)—Married Irish woman, thirty-eight years of age, without history or clinical evidence of syphilis and with a diagnosis of sciatica. Wassermann negative.

27. (244)—Single machinist, twenty-four years of age, without history or clinical evidence of syphilis. Wassermann negative.

28. (236)—Married tailor, fifty-one years of age, without history or clinical evidence of syphilis, but with keratosis. Wassermann negative.

29. (234) — Married woman, forty-seven years of age, without history or clinical evidence of syphilis, presenting arteriosclerosis and associated conditions. Wassermann negative.

30. (382)—Teamster, twenty-six years of age, with a history of syphilis and three injections of arsphenamine. Positive Wassermann.

31. (386)—Single Italian, thirty-one years of age, with a history of syphilis nine years ago, who has had a large amount of arsphenamine and mercury, but no positive Wassermann since 1920. Wassermann negative.

32. (384)—Married woman, sixty-one years of age, whose husband died of shock at forty-eight, who has had eight positive Wassermanns in the last two years and intensive treatment with arsphenamine and mercury. Diagnosis, treated syphilis. Wassermann doubtful.

33. (394) — Married teamster, thirty-eight years of age, without history or physical signs of syphilis; has boils. Wassermann negative.

34. (385)—Telephone operator, twenty-one years of age, with a history of syphilis five years ago and treatment; has had no positive Wassermanns in the last two years. Diagnosis, treated syphilis. Wassermann negative.

35. (383) — Negro, navy yard helper, nineteen years of age; has had gonorrhoea, but no history of syphilis. At present has scabies. Wassermann negative.

36. (398)—Italian married woman, thirty years of age, without history or symptoms of syphilis. Diagnosis, rheumatism. Wassermann negative.

37. (410)—Married teamster, fifty-six years of age, without history or clinical evidence of syphilis; now suffering with obesity and hypertension. Wassermann negative.

38. (389)—Italian barber with a history of syphilis and six positive Wassermanns and three negative Wassermanns.

39. (396)—Married Canadian woman, forty-two years of age, without history or clinical evidence of syphilis. Diagnosis, scabies. Wassermann negative.

40. (388) — Married Canadian woman, twenty years of age, without history or clinical evidence of syphilis. Diagnosis not given. Wassermann negative.

41. (415) — Jewish housewife, forty-four years of age, without history or clinical evidence of syphilis; suffering from obesity and constipation. Wassermann negative.

42. (390) — Married Jewess, twenty-nine years of age. Has husband and one baby with positive Wassermanns; she has had intensive treatment with arsphenamine and mercury; several positive Wassermanns, none positive, however, since 1920.

43. (411) — Salesgirl, sixteen years of age, without history or clinical evidence of syphilis, but who has neurasthenia. Wassermann negative.

Results.—Out of a total of 258 tests there are, then, but six flat disagreements, or 2.3 per cent., all but one of which are on cases of treated syphilis.

There are eleven cases (4, 6, 8, 9, 14, 16, 17, 19, 34, 36 and 42) in which one or more workers noted a tendency towards false positive results with the use of cholesterinized antigens, especially the State antigen, but in most instances there was sufficient evidence from other antigens to disregard these findings or to qualify them to the extent of giving a doubtful or negative report.

Acetone insoluble antigen with .1 cc. serum tends to give false negatives. (Cases 3, 5, 12, 13, 32 and 38.) With .4 cc. serum this is largely corrected. (Cases 3, 5, 12, 32 and 38.)

It is interesting to note how much closer readings are given with cholesterinized antigens when the two doses of serum, .1 and .05, are used as by Laboratory C.

On the whole, the tests indicate a high degree of accuracy in diagnosis, and strong agreement among different workers with the same specimens.

PART 3.

MILK COUNTS.

A COMPARISON OF REPORTS FROM SEVERAL LABORATORIES ON IDENTICAL SPECIMENS.

The bacterial examination of milk has become one of the most important of health laboratory tests. However experts may disagree as to the healthfulness or harmfulness of bacteria in milk, there remain certain important facts which cannot be denied. All other circumstances being equal, milk produced under sanitary conditions contains fewer bacteria than that produced under unsanitary conditions. Milk kept cold contains fewer bacteria than milk not kept cold, and fresh milk contains fewer bacteria than stale milk.

The milk bacterial count, then, is an index of the cleanliness of production, the proper cooling of the product and of its freshness, and a high count indicates a failure in one or all three of these conditions.

Considerable criticism has been brought against the bacterial count of milk because of the varying results obtained when split specimens have been submitted to different laboratories, and it is well known that variations in the media used and in the technique will produce wide divergence in the results.

The object of this series of tests was, first, to learn the technique in use in the various laboratories in this vicinity and to compare the results obtained, then, if possible, to adopt uniform technique and again compare results.

Twelve laboratories agreed to coöperate in milk counts, but because of the distance of some of these from Boston the work was carried on for the most part by but nine.

The twelve laboratories are as follows: Boston Health Department, Brookline Health Department, Brockton Health Department, Somerville Health Department, Wellesley Health Department, Alden Brothers, Boston Biochemical Laboratory, C. Brigham Company, Harvard Medical School Bacteriological Laboratory, H. P. Hood & Company and D. Whiting Company.

Questions concerning the technique in use in these laboratories were answered as shown in the following tables (pp. 12 and 13).

The principal point of difference in the technique is in the composition and reaction of the media used.

For the first and second series of tests six specimens each were submitted, care being taken to keep them well iced and to deliver to the laboratories as quickly as possible. The results are shown in Tables I. and II.

Discussion of Milk Tests.—Tables 1 *and* 2.—Table No. 1 shows what discordant results may be expected when counts are made with varying media and technique. Laboratory D reported such consistently abnormal low counts that inquiry was made into the method of media making, and it was discovered that on account of an error in titration calculation, which evidently had been followed for years, this laboratory was working with an alkaline medium.

Other counts are so far wrong as to indicate serious technical error. For instance, Laboratory F's report on Samples 1 and 2, or Laboratory I on Sample 6.

Wide as the variations are, however, if we consider the results only in relation to the Boston Health Department standard of 500,000 to a c.c., it will be found that out of fifty-four reports but seven are in error, and three of these are from Laboratory D.

Table No. 2 shows the results of another series of tests. In this series there is a closer approximation of similar conditions since several laboratories obtained media from the Boston Health Department Laboratory.

Laboratory F again reports counts on two samples, 5 and 6, which are difficult to explain, except by errors in technique. Laboratory D shows a similar report on Sample 2, and Laboratory A on Sample 4.

Judging the fifty-four reports from the 500,000 per c.c. standpoint, but two errors are shown: Sample 4 by Laboratory A, and Sample 5 by Laboratory F.

Technique for Milk Counts.—At a conference with seven of the coöperating laboratories represented it was unanimously agreed that a more uniform technique should be established and it was advised that laboratories of this vicinity use for the present as a medium for milk counts a meat-juice medium rather than one made with beef extract or other substitute; that this medium contains 1.2 per cent. agar, and, until such time as the laboratories shall become familiar with the hydrogen-ion titration of medium and shall agree upon the optimum reaction, the reaction shall be established by titration, using

TECHNIQUE IN USE FOR BACTERIAL EXAMINATION OF MILK SAMPLES.

Laboratory	Miscroscopic estimate of bacteria	Method	Percent of plating saved	Percent of agar used for plates	Reaction of media	Meat Juice or Beef Extr
A	at times, not often	Slack	0	1-1/2 %	+.5 Fuller's scale	Beef Extr
B	Yes	"	0	"	+.5 to +1.	Meat Juice
C	"	"	Raw Milk 75%	1%	+1.5	"
D	"	Slack Breed	20-25%	"	+1.	"
E	"	Slack	0	"	-	Beef Extr or Dehydrated
F	"	Slack Breed	90%	"	+ 1.5	Dehydrated
G	No	-	-	1.2 to1.5%	+.5 to +1.	Beef Extr.
H	Yes	Slack	0	1.2%	+1.4 or +1.5	Meat juice
I	No	-	-	1%	+1.5	"
J.	Yes	Slack	0	"	+1.5	"
K	"	"	33%	"	+1.5	"
L	No	-	-	1-1/2%	+1. to+1.5	Beef Extr

TECHNIQUE IN USE FOR BACTERIAL EXAMINATION OF MILK SAMPLES

Laboratory	Dilutions plated	Temperature of Incubation	Length of Incubation	Use of magnifying glass	*see foot-note
A	1-100 1-1,000 1-10,000 1-1,000,000	37-1/2°	48 hours	yes,4in	yes
B	1-100 1-10,000	"	24 & 48 hrs	yes,2-1/2	"
C	1-100 1-10,000 1-1,000,000	"	48 hours	yes	"
D	1-50 1-2,500	"	"	"	"
E	1-100 1-10,000		24 & 48 hrs	"	I think so
F	1-100 1-1,000 1-10,000	37-1/2°	48 hours	"	yes
G	1-100 1-10,000	"	"	"	not important if Technique is uniform
H	1-100 1-1,000 1-10,000 1-1,000,000	"	"	sometimes	Does not care to answer
I	1-100 1-10,000	37°	"	yes	yes
J	1-10,000 1-1,000,000	37-1/2°	"	"	"
K	1-100 1-1,000 1-10,000	"	"	"	yes if uniformity can also be had
L.	1-100	"	"	"	yes

* In agreeing upon a technique to be accepted by all the laboratories in this vicinity are you in favor of the technique which will give, as nearly as possible, the actual number of bacteria present?

Milk Counts

Table No. 1

Lab.	Dil.	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
A	Est	Occ. Org.	2,300,000	Occ. Org.	Occ. Org.	Millions	Millions
	1-100	3,300	336,000	8,200	spreader	too num.	too num.
	1-10,000	0	260,000	30,000	10,000	1,940,000	1,340,000
	1-10,000	10,000	350,000	80,000	spreader	1,380,000	1,500,000
B	1-100	2,400	Uncount.	90,000	40,000	Uncount.	
	1-10,000	0	1,140,000	155,000	450,000	"	7,000,000
C	Est.	30,000	1,000,000	200,000	60,000	5,000,000	2,000,000
	Report	15,000	2,150,000	180,000	45,000	9,250,000	4,270,000
D	Est.	5,000	5,000	5,000	5,000	15,000	15,000
	1-100	0	16,000	10,000	10,200	80,000	130,000
	1-10,000	0	20,000	0	10,000	90,000	120,000
E	Est	O.K.	O.K.	Pus	O.K.	O.K.	L.G.I.
	1-100	5,500			26,000		
	1-10,000		2,900,000	120,000		1,800,000	1,100,000
F	Report	1,600,000	250,000	50,000	40,000	2,500,000	1,500,000
G	Est	Few	Many	Few	Few	Many	Many
	Report	5,200	2,330,000	62,000	24,000	18,000,000	10,800,000
H	Est	Few	Many + Strep.pres.	Mod.	Mod.	Many+++	Many+
	1-1000	5,000	+++	spreader	12,000	++++	spreader
	1-10,000	20,000	1,570,000	10,000	40,000	Est 4,000,000	1,860,000
I	Report	20,000	680,000	10,000	30,000	Unsatisfactory	80,000

Milk Counts

Table No. 2

Lab.	Dil	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
A	Est.	Very few	Very Few	Over a Million	Over a Million	Medium Clean	Very Few
	1-100	28,000	spreaders	Uncount.	154,000' Spr	70,000Spr	1,500
	1-10,000	30,000	40,000	3,100,000	80,000	220,000	2,000
B	Est	No.bact.	No bact.	Many bact.	Many bact	Few bact	no bact.
	Report	4,000	70,000	2,000,000	1,600,000	70,000	4,000
C	Est	50,000	90,000	7,000,000	4,000,000	200,000	20,000
	1-100	18,200	48,000	Innumer.	Innumer.	spreader	6,000
	1-2000	34,000	60,000			250,000	4,000
	1-2,000	30,000	68,000			258,000	6,000
	1-10,000			9,000,000	2,500,000		
	1-10,000			9,600,000	2,340,000		
	Report	18,200	48,000	9,300,000	2,420,000	254,000	5,000
D	Est.	30,000	5,000	2,500,000	1,500,000	230,000	0
	1-100	40,300	2,100	Uncount.	700,000	spreader	3,600
	1-10,000	60,000	0	2,000,000	800,000	410,000	10,000
E	Est	O.K.	O.K.	L.I.	O.K.	O.K.	O.K.
	1-100	53,200					3,500
	1-100	36,400					3,300
	1-10,000		230,000	2,500,000	9,500,000	310,000	
	Report	45,000	230,000	2,500,000	9,500,000	310,000	3,400
F	Report	40,000	250,000	13,500,000	9,600,000	900,000	100,000
G	Est	Few	Numerous	Uncount.	Many	Numerous	Some Clumped
	Report	20,000	68,000	870,000	4,700,000	110,000	3,600
H	Est	Few	Few	Many+	Many-	Mod	Few
	1-100	21,000	12,000	+++	++	37,000	5,100
	1-10,000	40,000	30,000	2,790,000	600,000	60,000	10,000
I	1-100	32,000	121,000	Uncount	Uncount	78,200	4,800
	1-10,000	30,000	110,000	5,850,000	6,800,000	310,000	20,000

Milk Counts

Table No. 3

Lab.	Dil.	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
A.	1-100	29,500	1,300	2,600	Over 2½Mill.	46,800	3,400
	1-10,000	120,000	0	0	"	490,000	90,000
B.	Est.	Few	0	Few	Many	Few	Few
	Report	250,000	2,550	21,100	Over 2½Mill.	110,000	23,000
C.	Est.	150,000	10,000	20,000	Over 2 Mill.	60,000	100,000
	1-100	110,000	1,400	2,000	Innum.	37,000	25,700
	1-100	100,000	1,600	1,600	Innum.	36,400	26,500
	1-10,000	100,000	0	0	2,800,000	40,000	20,000
	1-10,000	120,000	0	0	2,700,000	30,000	50,000
	Report	110,000	1,500	1,800	Over 2½Mill.	36,700	26,100
D.	Est.	110,000	10,000	30,000	Over 2½Mill.	30,000	20,000
	1-100	38,000	8,000	11,000	"	33,000	19,000
	1-10,000	140,000	10,000	50,000	"	50,000	20,000
E.	Report	205,000	285,000	5,500	"	45,000	50,000
F.	1-100	120,000	5,000	10,000	"	100,000	50,000
	1-10,000	250,000	10,000	20,000	"	150,000	70,000
G.	1st Dil.	70,000	3,500	40,000	"	95,500	67,000
	2nd Dil.	60,000	10,000	30,000	"	90,000	60,000
H.	Est.	Few	Few	Few	Many +++	Few +	Few
	1-100	130,000	2,600	6,100	++++	34,600	65,000
			2,300				
	1-10,000	130,000	0	10,000	Over 2½Mill.	60,000	60,000
			0				
I.	No report received from this laboratory.						
J.	Report	180,000	2,700	5,800	Over 2½Mill.	180,000	23,000

phenolphthalein as an indicator, and shall be +1.5 Fuller's scale.

Plates shall be incubated forty-eight hours at 37° C. in an incubator containing water.

Earthenware tops to Petri dishes may be used in preference to glass tops, in order to control spreaders as far as possible.

Dilution water bottles must be filled sufficiently to overcome the loss by evaporation during sterilizing.

It was also agreed that as soon as time had been allowed for the various laboratories to conform to these requirements another series of tests would be run, each laboratory to report on but two dilutions, 1-100 and 1-10,000, all 1-10,000 plates containing over 250 colonies to be reported "over 2,500,000."

The results of this third series of tests appear in Table No. 3.

While some returns in this report indicate technical errors (Laboratory E report on Specimen 2 and Laboratory A on Specimen 5—1-10,000 dil.), the returns as a whole are very uniform and show what can be done when like technique and media are used.

Considered from the standard of 500,000 to a c.c., there are no errors in this report, but every worker agrees on every sample.

Some such uniform methods should be universally adopted, methods which, as nearly as possible, will give the actual number of bacteria present per cubic centimeter in the sample tested.

PART 4.

Technique for Health Department Diagnostic Tests.—These notes on technique are simply the expression of opinion of the majority of those engaged in the comparative tests previously reported. Several conferences of these workers were held for discussion of the results obtained and for the adoption of more uniform procedures. No claim is made for originality.

DIPHTHERIA.

Staining—Loeffler's alkaline methylene blue is recommended for routine work. Slides should be cold and stain applied for not more than one-half minute. Kinyoun's stain is also recommended; slides cold, stain applied two minutes.

Stains: *Loeffler's Alkaline Methylene Blue.*

Stock sol.: Methylene blue, 1 part; 95 per cent. alcohol, 10 parts.

Stain: Stock sol. 300 c.c. 1-10,000, KOH 1000 c.c.

KINYOUN'S STAIN.

Toluidin Blue	0.5 gr.
Azur ii	0.05 gr.
Meth. Blue	0.05 gr.
Alcohol (95%)	25.00 c.c.
Acetic (glacial)	1.00 c.c.
Distilled water to	600.00 c.c.

Dissolve stains in alcohol, add water, add acetic last.

Since some of the laboratories are experimenting with Dr. Albert's stain, the formula is given here, and other laboratories are asked to compare it with the stain in regular use. This applies also to Dr. Kinyoun's stain. It is urged, however, that no new stain, however good, be adopted for routine work until the technician has become thoroughly familiar with it through a long series of comparisons with the stain to which he is accustomed.

ALBERT'S STAIN.

Solution 1.

Toluidin Blue	0.15 gm.
Methyl green	0.20 gm.
Acetic acid (glacial)	1.00 c.c.
Alcohol (95%)	2.00 c.c.
Water (distilled)	100.00 c.c.

After standing for one day, the solution is filtered and is ready for use.

Solution 2.

Iodin	2 gm.
Potassium iodid	3 gm.
Water (distilled)	300 c.c.

The solution is ready for use as soon as the iodin is dissolved entirely. The directions given by Dr. Albert are as follows: "Smears are made on slides or cover-glasses; fixed by heat; stained with Solution I for one minute; washed with water; dried with good absorbent filter paper; stained with Solution II for one minute; washed and dried with filter paper. The granules of the diphtheria bacillus are stained black, the bars dark green and the intermediate portions a light green. Virtually all other bacteria also take a light green stain."

Diagnosis.—Except in diagnosis of young cultures diagnosis should be made only on A, C and D types "Wesbrook."

When other of the major forms are present, as A1, A2, B, B1, B2, C1 or C2, but no granules can be demonstrated, a report of "suspicious" should be made and another culture requested. Forms lower in the scale than D may be disregarded. Typical grouping and irregularity of form shall also be considered, but diagnosis should not be made on these points unsupported by the finding of granules. These rules apply also to swab examinations.

In examinations of young cultures the so-called clove-forms with unstained septum and without granules are diagnostic.

An assistant should not be permitted to give an independent report on cultures without proven ability and should also have at least six months' training in routine work in a laboratory handling many cultures. For reports on swabs and young cultures the training should extend over at least a year. When two or more diagnosticians are available, reports should be made as agreed upon by two.

Medium.—Loeffler's blood serum. (Horse, beef or pig serum may be used.)

Incubation.—Fourteen or 15 hours at 37° C. is recommended. For the examination of young cultures five hours' incubation or over, the cultures may be removed but should be replaced for the full period of incubation, and examination made again.

Smears.—Smears should be made representative of the whole growth and obtained by rubbing the platinum loop over the whole surface of the serum.

The material thus obtained should be rubbed, with a drop of fresh tap water, in a thin smear over a glass slide, the smear covering an area of at least 1 inch by one-half inch.

A number of such smears on a large glass slide is allowable, provided care is taken to separate the preparations with wax pencil marks and not to allow spattering from one preparation to another.

Cultures which show liquefaction or sufficient contamination to prevent a diagnosis, or which are excessively dry, shall not be considered satisfactory for examination if the result is negative.

Reports.—The reports of the examination shall be:

1. Diphtheria bacilli found.
2. Diphtheria bacilli not found.
3. Culture contains suspicious forms.

When suspicious forms are found a swab preparation shall be examined, if this has not been done. The culture shall also be reinoculated on fresh media and reincubated for further study.

4. Culture unsatisfactory, contaminated or dry. In the latter two cases, 3 and 4, another culture shall be requested. Another culture shall also be requested when the result of the examination is negative, if the physician's diagnosis is diphtheria, or if it is stated that the inflammatory reaction is in the larynx.

The organisms associated with Vincent's angina, as found in swab examinations, or the *unusual* predominance of streptococci either in swabs or culture, shall be reported when found.

If there is a suspicion of Vincent's angina a swab preparation stained deeply with methylene blue shall be made.

Virulence Tests.—Virulence tests on all clinical cases shall be run on request, provided the patient has been quarantined three weeks or over. The State laboratory prefers a period of six weeks' quarantine.

Virulence tests may be made when typical morphological organisms are found and the patient has shown no symptoms of the disease. For the virulence test three or more colonies of the organism shall be isolated and grown in bouillon, + 1 Fuller's scale, for either twenty-four or forty-eight hours. Do not filter. A day's time may frequently be saved in giving a positive virulence report by use of the twenty-four-hour culture. The culture should, however, be further incubated and if the results of the first inoculation are negative after twenty-four hours, a second pig should be inoculated with the forty-eight-hour culture. A control animal inoculated with the same dosage of toxin, but protected with antitoxin, may be used if desired. For dosage use one-half of one per cent. of the body weight of the guinea-pig, injecting subcutaneously in the abdominal wall. Report positive on death of the guinea-pig or if there is any marked inflammatory reaction in twenty-four hours or more and if autopsy shows marked inflammation of the supra-renal capsules. A negative report shall not be made until at least forty-eight hours after the inoculation.

TUBERCULOSIS.

Rules agreed upon are as follows:

Specimens shall be accepted only in outfits provided by the laboratory.

Specimens which have leaked or run over the sides of the container will not be accepted.

Container should have at least one-fourth volume of an efficient disinfectant.

The outside of the container, box, slip, etc., should be disinfected after receipt at the laboratory.

Sputum should be prepared for examination by shaking, digesting with antiformin, or by a combination of both these processes.

Smears should be at least one inch by one-half inch in area.

For staining, Ziehl's carbol-fuchsin is recommended, applied for five minutes steaming; decolorize with alcohol (denatured or 70 per cent. grain) containing 3 per cent. hydrochloric acid. Other accepted decolorizing agents may be used if preferred. Counterstain with methylene blue.

A positive sputum shall be used as a control. The absence of tubercle bacilli shall be reported only after the specimen has been examined for at least five minutes.

A positive diagnosis shall not be made unless at least ten acid-fast bacilli are found, or unless the bacilli are found in two preparations.

Sputum containers shall be used but once and destroyed.

Slides or centrifuge tubes, if used, shall be cleansed by immersion in sulphuric acid and potassium bichromate for at least twenty-four hours before being used again.

In the examination for tubercle bacilli of specimens other than sputum, such as urine or pus, the finding of acid-fast organisms, or their absence, shall not be considered sufficient evidence for diagnosis, but guinea-pig inoculations shall be made.

Guinea-pig inoculations are made by subcutaneous inoculation of the suspected material in the abdominal wall. The animal should be kept six weeks under observation, then chloroformed and autopsied, a positive diagnosis being made on finding the bacilli in the inguinal glands, spleen or other glands or organs. Animals may be autopsied earlier if enlarged glands are present.

Technique, Widal Test.—Specimens of dried blood which are used in making the agglutination test shall be diluted approximately 1-25 with water or physiological salt solution.

A motile culture of the typhoid bacillus in bouillon which agglutinates readily with positive serum shall be used for the test.

This bouillon culture should be made from a stock agar culture which has been attenuated by storage, for about one month, on ice.

The stock agar culture is kept by transplants from agar to agar.

The bouillon culture should be incubated at a temperature between 20° C. and 37° C. for not more than eighteen hours. The culture shall not be disturbed, and only the surface shall be used for the test; or if the growth is too heavy it may be diluted to correspond to a turbidity standard.

One loop of this culture and one loop of the 1-25 dilution of blood are mixed on a cover-glass, and a hanging-drop preparation is made which should be examined at once, and again after one hour. A hanging-drop of the culture alone should also be made and used as a control.

The diagnosis of typhoid fever shall be made on complete agglutination and loss of motility in one hour. If the reaction is partial, it should be so reported and another specimen requested.

Positive results on specimens from vaccinated persons are unreliable.

Repeated macroscopic tests, for observation of change in the agglutination titre, are recommended for the diagnosis of typhoid fever in those who have been vaccinated.

GONORRHOEA.

Technique.—Smears are stained by Gram's method, and the diagnosis is made on the presence of gram-negative intracellular diplococci of typical shape and grouping in the pus cells.

In chronic cases a positive finding may be made on the presence of typical organisms in shreds or in epithelial cells.

In any case where such organisms are found the report shall be "Gram-negative, intracellular diplococci present."

Stains, Sterling's Method.—Two c.c. anilin oil + 10 c.c. 95 per cent. alcohol. Shake and add 88 c.c. distilled water.

Five grams of gentian violet are ground in a mortar and the anilin solution added slowly while grinding. Filter.

Directions for Staining.—Sterling's solution of gentian violet, cold, one-half to one minute. Wash. Gram's iodin solution, one minute. Wash. Decolorize with alcohol. Wash. Counterstain with bismarck brown, weak carbol-fuchsin, 2 per cent. aqueous pyronin or other weak solution of a contrasting dye.

Carbol Gentian Violet.—Saturated alcoholic solution of gentian violet, 90 c.c. Five per cent. phenol in water, 1000 c.c. This stain keeps well, but is not as permanent as Sterling's.

WEIGERT'S METHYL VIOLET

Solution 1.	Absolute Alcohol, 33. Aniline oil, 9. Methyl violet in excess.
Solution 2.	Saturated aqueous solution of methyl violet.

The staining solution consists of:
Solution 1. 1 part.
Solution 2. 9 parts.

This mixture will keep at the most for fourteen days.

Burke's Stain (Jour. A. M. A., September 24, 1921). 1. Flood slide with anilin gentian violet or a 1 per cent. solution of aqueous solution of methyl violet. Thoroughly mix with the dye on the slide a few drops, three to eight, depending on the amount of dye, of a 5 per cent. solution of sodium bicarbonate. Allow to stand for two or three minutes.

2. Flush off the excess stain with iodin solution (1 gm. of iodin, 2 gm. of potassium iodid and 100 c.c. of distilled water) and cover with fresh iodin solution and allow to stand one minute or longer.

3. Wash in water as long as desired and blot off all free water until the surface of the film is practically free of water, but do not allow the film to become dry. The success of the stain depends largely on the proper control of this step.

4. Decolorize with acetone or acetone and ether (one part of ether to from one to three parts of acetone) until the acetone flows from the slide practically uncolored. This usually requires less than ten seconds. The acetone should be placed on the slide. The slide should not be dipped in the decolorizer.

5. Blot dry. The slide quickly dries without blotting.

6. Counterstain for five or ten seconds with a 2 per cent. aqueous solution of safranin O. Exposure to the counterstain can be increased, depending on the excellence of the violet dye used.

7. Wash off excess stain by short exposure to water; blot and dry.

8. Immerse in xylene or turpentine for several minutes, or until clear. Examine.

Technique of Wassermann Test.—For discussion of the Wassermann work as a whole, including all the comparative tests done, it was found difficult to arrange meetings suited to the time of all the participants. Those who were able to be present at these discussions agreed on the use of the technique advised by the State Wassermann Laboratories, using sheep-cells, anti-sheep cell amboceptor and for complement guinea-pig serum.

The reagents are to be prepared and standardized in accordance with the technique of the State Laboratory (*American Journal of Syphilis,* October, 1920).

Minor differences in technique seem to be unavoidable. The exact volume of the test is not important, the majority preferring a volume of 2.1 c.c.

While pooled serums from several guinea-pigs is advised for complement, there is no objection to the use of serum from a single pig, provided it titres correctly.

It is advisable while complement is out of the ice chest, during the tests, to keep it cold by placing it in an iced receptacle.

It is advised that laboratories using the State antigen control it with at least two others, either two cholesterinized antigens of different strength or one cholesterinized and one acetone insoluble antigen. When an acetone insoluble antigen is used the dosage of serum should be .4 c.c. and a control should be run with an equal amount.

No antigen should be used in quantity exceeding one-fourth of the anticomplementary dosage.

The method of dilution of an antigen is of great importance, and differences in the technique in this dilution may account for false end-results in the test.

In diluting the State antigen the salt solution should be added through a special pipette, prepared to deliver slowly, until a quantity equal to the amount of antigen has been added, after which the amount necessary to complete the dilution is added as quickly as possible. During the first part of the process the mixture is constantly agitated.

It has been promised that as soon as possible a State antigen will be provided with sufficient antigenic power to allow a larger anticomplementary margin, and that accurate antigen dilution pipettes, with directions for their use, will be furnished each laboratory using the State technique.

In reporting results the following terms shall be used: "Positive," "Negative," "Doubtful," "Unsatisfactory." If desired, a positive report may be further characterized as strongly, moderately or weakly positive. Explanation of an "Unsatisfactory" report may be given as "Anticomplementary," "Hemolysed" or "Bacterially contaminated."

DISCUSSION.

In discussing the whole investigation, the writer wishes to express his appreciation of the cordial coöperation of all the laboratories represented and the great interest shown in the comparative tests. Much would be gained in accuracy by the uniform adoption of standard procedures; that is to say, by an agreement to adopt the best methods.

There is a tendency in any laboratory doing to lose tone. A series of comparative tests routine work to get into a rut, so to speak, and awakens the interest and stirs the worker to a careful observance of his technique. Errors in technique which may have been long unrecognized are revealed and corrected and the conferences of technicians from various laboratories tend to introduce more generally improved technical methods. In the Wassermann work the tests with serums from cases in which the clinical history is compared with the findings has been especially helpful in showing the value of the reactions. Accuracy in laboratory tests is indispensable, and it would be of great benefit in this regard if some arrangement could be made for a constant check or control on diagnostic work, either by the submitting of specimens for comparative tests, by submitting, without the knowledge of the technician, known positives or negatives, or by observing the technique and checking the regular routine.

The smaller laboratories labor under a serious handicap, since they deal with but few specimens, and, because of lack of practice, may make erroneous returns. A false negative on a case of diphtheria or a false positive on a Wassermann may, either one, mean serious trouble. If city laboratories where technicians must pass civil service examinations are not free from error, what shall be said of private laboratories, which are under no restrictions?

Would it not be a good plan to require that all persons who assume the responsibility of reporting on diagnostic tests should pass an adequate examination and should display certificates in their laboratories?

For oversight of the technique of smaller laboratories and for help in their problems a number of towns and cities might associate in the employment of an expert, or such a person might be engaged by some central authority, and his services given to those laboratories requesting the same; his duties to consist in visiting the various laboratories, observing the technique, advising or suggesting improvements, and checking the diagnoses given, to study new technique and to introduce improved methods, to report any marked inefficiency, and in time of emergency, as in an epidemic, to supplement the work of the regular technicians.

Those engaged in the bacterial examination of milk, which is conceded by most public health authorities to be much more important than the chemical tests, should be required to pass examination before a duly qualified board of examiners and should have their laboratories visited and their technique checked, as is now required for those doing the Babcock test.

Private laboratories offering services in diagnosis tests should also come under sufficient expert supervision to safeguard the public.

The diagnostic laboratory has been of incalculable service, and its importance will increase rather than decrease in the years to come. It is to the advantage of all concerned that all such laboratories be maintained at a high point of efficiency.

CONCLUSIONS.

The best approved methods should be agreed upon and adopted as standards by all diagnostic laboratories.

Those engaged in laboratory diagnostic work should have adequate training and should be of proved ability.

Some method of supervision or of mutual association of diagnosticians should be devised to insure the use of uniform technique and as a control on the accuracy of the work.